

CLAIMS

1. Method for the demonstration of the occurrence of a specific molecular event in a cell,
5 wherein:

- the "solubilization" of a "bound" marker protein (respectively the "binding" of a "solubilized" marker protein) that is a direct or indirect marker for the occurrence of the specific molecular event is
10 detected,

- said marker protein is present in the cell before the aforementioned detection,

- the cell, before the detection, is subjected to a permeabilization of the plasma membrane which
15 releases the solubilized protein into the extracellular medium,

- the presence of the marker protein is then detected in the cell or in the extracellular medium by any appropriate means that makes it possible to
20 determine if solubilization, respectively binding, has occurred, and thus the corresponding molecular event.

2. Method according to claim 1, wherein the cellular "binding" of the protein is carried out by way of subcellular anchoring, preferably membrane
25 anchoring, of the protein, or by compartmentalization of the protein at the subcellular level.

3. Method according to one of the claims 1 and 2, wherein the cellular solubilization of the protein is obtained by release of the marker protein in the
30 cytosol.

4. Method according to one of the claims 1 to 3, wherein the marker protein is a fusion protein containing a fluorescent fragment.

5. Method according to one of the claims 1 to 4, wherein the marker protein is produced in the cell by an expression vector.

6. Method according to one of the claims 1 to 5, wherein the marker protein is constitutively produced by the cell.

7. Method according to one of the claims 4 to 6, wherein solubilization, respectively binding, of the fluorescent protein is detected by flow cytometry or fluorescence microscopy on the cells after permeabilization of the membrane.

8. Method according to one of the claims 1 to 6, wherein the occurrence of the molecular event leads to the cleavage or the modification of the marker protein and solubilizes it.

9. Method according to one of the claims 1 to 6, wherein the occurrence of the molecular event leads to the appearance of a subcellular anchoring fragment, preferably a membrane anchoring fragment, of the marker protein and to its binding, or to the compartmentalization of the marker protein.

10. Method according to one of the claims 1 to 9, wherein the molecular event to detect is Bax activation, wherein the marker protein is a Bax-fluorescent protein fusion protein, the fluorescent protein being fused at the N-terminal end of Bax, and wherein, in the event of Bax activation, the Bax protein is bound.

11. Method according to one of the claims 1 to 9, wherein the molecular event to detect is the activation of a protease, wherein the marker protein is a fusion protein containing the protease cleavage site and, on either side, a subcellular anchoring site, preferably a membrane anchoring site, and a fluorescent protein, and wherein, when the protease is expressed, the marker

protein is solubilized by cleavage and the fluorescent protein is released.

12. Method according to claim 11, wherein the protease is a caspase.

5 13. Method according to one of the claims 1 to 12, wherein the demonstration of the occurrence of the molecular event is coupled with the measurement of the cell cycle, in particular the measurement of the distribution of the cell population in the various
10 phases of the cell cycle.

14. Method according to claim 13, wherein the molecular event is the activation of Bax or the activation of a caspase.

15 15. Marker protein for use in the method according to one of the claims 1 to 14, wherein it contains a sensing component which will undergo solubilization (binding) and an indicator component that enables detection.

20 16. Protein according to claim 15, wherein it is a fusion protein whose indicator component is a fluorescent protein.

25 17. Marker protein according to claim 15 or 16, wherein the sequence of said sensing component is coded by a nucleic acid comprising a sequence chosen among sequences SEQ ID Nos. 1, 3, 5, 7, 9, 11, and 13.

15 18. Marker protein according to one of the claims 15 to 17, wherein the sequence of said sensing component includes a sequence chosen among sequences SEQ ID Nos. 2, 4, 6, 8, 10, 12, and 14.

30 19. Vector expressing, in a cellular environment, a marker protein according to one of the claims 15 to 18.

20. Transformed cell expressing a marker protein according to one of the claims 15 to 18.

21. Cell according to claim 20, wherein the expression of the marker protein is stable.

22. Cell according to the claim 20 or 21, wherein it is a tumor cell, preferably a human tumor cell.

5 23. Non-human transgenic animal in which at least a certain type of cells expresses a marker protein according to one of the claims 15 to 18.

10 24. Kit for implementing the method according to one of the claims 1 to 14, wherein it contains at least:

- cells transformed according to one of the claims 20 to 22; and/or
- a vector according to claim 19; and/or
- a transgenic animal according to claim 23.

15 25. Method for evaluating the activity of a candidate anti-cancer compound, wherein it includes the implementation of the method according to one of the claims 1 to 14 in a cell according to claim 22.